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Mini Review

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COVID-19: Neuroinvasiveness, Neurotropism and Neurovirulence

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Abstract

Neurological complications have emerged as a significant cause of morbidity and mortality in the ongoing COVID-19 pandemic. In this focused review, we summarize evidence of the neuroinvasiveness, neurotropism and neurovirulence of the novel beta coronavirus SARS-CoV-2. Potential means of neuroinvasion include hematogenous spread, neuronal retrograde transport from the vagus or olfactory nerves and the transcribrial route. Pathologic studies suggest direct neuroinvasion *via* hematogenous spread and retrograde transport by the olfactory nerve, while retrograde transport through the vagus and olfactory nerves remains hypothetical. Experimental evidence confirms that angiotensin converting enzyme 2 (ACE2) is the main receptor for SARS-CoV-2, suggesting this ACE2 as a target of neurotropism. Direct evidence of detection of the virus in cerebral spinal fluid or post-mortem brain tissue is sparse. There is a paucity of reported post-mortem neuropathological examinations in victims of COVID-19, highlighting the importance of accruing additional cases. The potential for long-term neurological consequences of COVID-19 signals the importance of continued surveillance for neuroimmune disorders and neurodegenerative in those infected by SARS-CoV-2.

Keywords

COVID-19, SARS-CoV-2, Central nervous system, Infection, Inflammation

Introduction

Coronavirus disease 2019 (COVID-19) is caused by a novel human coronavirus, formally designated severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2). As of November 11, 2020, this pandemic has caused 51.9 million confirmed cases worldwide, with approximately 1.28 million deaths [1]. In the months since the start of the pandemic, COVID-19 has ravaged health and economies on all continents except Antarctica. At the time of this writing, there are no signs of pandemic abatement. Emerging evidence additionally suggests delayed sequelae in both adults and children.

Human coronavirus has long been known to affect the central nervous system (CNS) [2]. Multiple reports have been published describing neurological manifestations in patients with COVID-19 [3-7, 8, 9]. Reported neurological manifestations include, but are not limited to headache, impaired consciousness, stroke, seizure, meningitis, encephalitis, necrotizing encephalopathy, Guillain-Barre syndrome and acute demyelinating encephalitis [3]. The prevalence of CNS complications in patients with COVID-19 is estimated to be 0.04-0.20%

based on previously reported cases in severe acute respiratory syndrome and Middle East respiratory syndrome [3]. These complications can be roughly grouped as due to direct CNS injury from the virus, immune-mediated disease and systemic effects of COVID-19 [3]. In this paper, we review direct evidence of the neuroinvasiveness, neurotropism and neurovirulence of SARS-CoV-2.

Evidence of Neuroinvasiveness, Neurotropism and Neurovirulence of Pre-COVID-19 Human Coronaviruses

Neuroinvasiveness refers to the ability of the virus to enter the nervous system [10]. The potential routes include hematogenous spread, neuronal retrograde transport and the olfactory transcribrial route. Hematogenous invasion has not yet been documented in human coronaviruses, despite the fact that SARS-CoV and other human coronaviruses are capable of infecting myeloid cells [11], suggesting a role of myeloid cells as a virus reservoir. Neuronal retrograde transport *via* olfactory transcribrial pathways has been reported in SARS-CoV [12].

Neurotropism describes the propensity of a pathogen to infect cells within the nervous system [10]. Once in the CNS, viral neurotropism becomes a factor of the interaction between the structural proteins of the virus and the binding receptors of the host.

Neurovirulence is the capacity of pathogens to cause disease of the nervous system [10]. Clinical evidence of the neurovirulence of coronaviruses is summarized in table 1. Human coronaviruses are capable of causing both acute and chronic neurological conditions [2]. The acute damage is due to direct infection of the virus and the chronic pathologies are likely mediated by the immune responses.

Additional mechanisms of neurologic injury by human coronaviruses include the potential for inducing acute or chronic neuroimmunologic activation. One case report documented human coronavirus present in a patient with acute disseminated encephalomyelitis [13]. Cerebrovascular abnormalities have been described in both SARS and MERS patients [14, 15]. However, unlike varicella-zoster viral infection, human coronavirus-induced arteritis has not been observed. Given that a large proportion of critically ill SARS patients develop venous thromboembolism [16], ischemic cerebrovascular events may be secondary to comorbid hypercoagulation [15] instead of direct neurovirulence. Chronic CNS damage from human coronavirus is postulated to be associated with human coronaviruses detected in brain parenchyma; coronavirus antibodies and RNAs have been demonstrated in the CSF of multiple sclerosis patients [17-21]. Taken together, these findings suggest the role of human coronavirus in both acute neurologic injuries and chronic autoimmune disorders of the CNS.

Neuroinvasiveness of SARS-CoV-2

During the current pandemic, multiple reports of COVID-19-associated neurological disorders have emerged. SARS-CoV-2s neurologic disorders likely have similar biological mechanisms as seen in SARS and MERS. As the neuroinvasiveness of SARS-CoV-2 depends on either hematogenous spread, neuronal retrograde transport, or transcribrial routes [5], SARS-CoV-2 seems to enter the CNS hematogenously with ease. Pathological studies have shown direct invasion of the novel coronavirus into vascular endothelial cells [6]. More specifically, one autopsy study found SARS-CoV-2 particles in the neurons and capillary endothelial cells in the frontal lobe of a COVID-19 patient [7].

The underlying mechanisms of the hematogenous spread are yet to be fully elucidated, however. The viral particles likely penetrate the cerebrovascular endothelium in the same fashion as the viremia distributes the virus throughout the body, presumably causing mother-to-fetus transmission [22]. SARS-CoV-2 can also trigger cytokine storms resulting in elevated production of proinflammatory cytokines and chemokines [23], presumably increasing the permeability of the blood-brain barrier (BBB). Another proposed mechanism of the hematogenous route is *via* infection of peripheral immune cells, also known as "Trojan horse" trafficking [8]. A recent study found highly expressed viral RNA sensor genes in peripheral blood mononuclear cells [24]. Post-mortem examination revealed positive SARS-CoV-2 RNA in macrophages of the spleen [25]. Unfortunately, to our knowledge, none of the studies have yet demonstrated an explicit causal relationship. Viremia and BBB disruption do not necessarily mean that the virus traverses the BBB. Similarly, RNAs in immune cells alone is not enough to prove active replication in these cells.

During this pandemic, the high prevalence of olfactory dysfunction suggests the potential for neuronal retrograde transport as a mechanism for neuroinvasion [26]. When the olfactory nerves pass through the cribriform plate, they also penetrate the subarachnoid space as a conduit to enter the CSF. This special access, the transcribrial route, has been described in drug delivery systems [27]. Alternatively, virus particles can presumably pass directly through the damaged endothelium [5]. To date only one pathological study demonstrated putative neuroinvasion of SARS-CoV-2s into the olfactory nerve. The authors observed progressively less severe distribution of damage from the olfactory nerve to the brain stem [28], further supporting retrograde transport of the virus. However, the finding is based solely on ultrastructural observations without further confirmatory identification of SARS-CoV-2 particles. Despite this ultrastructural evidence of SARS-CoV-2 in the olfactory nerve, more evidence is required to confirm the olfactory nerve as a route into the CNS. The presence of anosmia does not require invasion into the olfactory nerve. Preliminary studies showed that SARS-CoV-2 binding receptors ACE2 and transmembrane protease serine 2 are expressed more in the non-neuronal cells of the olfactory epithelium than in the neurons [29-31]. Therefore, anosmia could be due to the destruction of the epithelial integrity rather than direct infection of the olfactory nerve.

The enteric nervous system is another candidate for

Table 1: Acute CNS disorders with direct evidence of coronavirus in the CSF or brain tissue.

Virus species	CNS disorders	Demographics	CNS manifestations	Investigations of coronavirus	Investigations of other etiologies
Unspecified [71]	CNS Infection	Totally 22 children, 18 male, median age 36 months	Headache, vomiting, seizure, meningeal irritation signs, Babinski sign	Serum: anti-CoV IgM (+) CSF: anti-CoV IgM (+)	CSF: bacteria, fungus, or mycobacterium tuberculosis excluded
HCoV-OC43 [72]	CNS Infection	11 m/o, male	Irritability alternating with sleepiness and abnormal posturing movements	Brain biopsy: RT-PCR and immunohistochemical analysis (+)	CSF: extensive panel of PCR for viruses and bacteria (-)
HCoV-OC43 [4]	CNS Infection	17 m/o	Altered behavior, myoclonic seizures	Nasopharyngeal swab: RT-PCR (+) CSF: RT-PCR (-) Brain: next-generation sequencing (+)	CSF: PCR for HSV, VZV, enterovirus, EBV, cytomegalovirus, human herpesvirus 6, parvovirus B19, adenovirus, and influenza (-); cryptococcal antigen and Borrelia burgdorferi IgM/IgG (-)
HCoV-OC43 [13]	Acute disseminated encephalomyelitis	15 y/o, male	Hypoesthesia, paresis, ataxia	Nasopharyngeal swab: RT-PCR (+) CSF: RT-PCR (+)	CSF: bacteria culture (-)
SARS-CoV [73]	Encephalopathy	39 y/o, male	Progressive dysphoria, vomiting and delirium	Brain suspension: RT-PCR (+) and viral particles compatible with coronavirus	Not mentioned
SARS-CoV [74]	Encephalopathy	59 y/o, female	Status epilepticus	Serum: RT-PCR (+) CSF: RT-PCR (+)	CSF: bacteriologic and fungal cultures (-)
SARS-CoV [75]	Encephalopathy	32 y/o, female	Generalized tonic-clonic seizure	CSF: RT-PCR (+)	CSF: Gram stain, bacterial cultures, and viral cultures (-)
SARS-CoV-2 [54]	CNS Infection	24 y/o, male	Seizure, altered consciousness, vomiting and neck stiffness	Nasopharyngeal swab: RT-PCR (-) CSF: RT-PCR (+)	CSF: not mentioned
SARS-CoV-2 [60]	CNS Infection	40 y/o, female	Headache, seizure, altered consciousness, hallucination and neck stiffness	Nasopharyngeal swab: RT-PCR (+) CSF: RT-PCR (+)	CSF: bacterial culture and HSV-1 PCR (-)
SARS-CoV-2 [55]	CNS Infection	56 y/o	No published data	CSF: RT-PCR (+)	No published data
SARS-CoV-2 [56]	CNS Infection and intracranial hemorrhage	36 y/o, male	Headache, vomiting, altered consciousness	Nasopharyngeal swab: RT-PCR (+) Fluid from the chronic subdural hematoma: RT-PCR (+)	CSF: not mentioned
SARS-CoV-2 [61]	CNS Infection	47 y/o, male	Progressive vertigo, headache, and ataxia.	Nasopharyngeal swab: RT-PCR (+) CSF: RT-PCR (+)	CSF: Gram stain, culture (-); PCR for HSV, VZV, EBV, influenza virus (-); IgM/IgG for Borrelia (-); paraneoplastic and autoimmune antibodies (-) Serum: paraneoplastic and autoimmune antibodies (-)
SARS-CoV-2 [76]	Acute necrotizing encephalopathy	55 y/o, female	Altered consciousness, multifocal myoclonus	Nasopharyngeal swab: RT-PCR (+) CSF: RT-PCR (+)	CSF: PCR for HSV, VZV (-)
SARS-CoV-2 [62]	Acute disseminated encephalomyelitis	64 y/o, female	Headache, irritability, bilateral vision impairment, sensory level, Babinski sign	Nasal swab: RT-PCR (-) CSF: RT-PCR (+) Serum: anti-SARS-CoV-2 IgG (+)	Serum: antiaquaporin-4 and anti-myelin oligodendrocyte glycoprotein antibodies (-)

SARS-CoV-2 [57]	Clinically isolated syndrome	42 y/o, female	Sensory abnormalities	Nasopharyngeal swab: RT-PCR (-) CSF: RT-PCR (+)	CSF: not mentioned
SARS-CoV-2 [28]	Encephalopathy	54 y/o, male	Headache, possible seizure	Sample not specified: RT-PCR (+) Gyrus rectus and medulla: particles referable to virions of SARS-CoV-2	Not mentioned

Abbreviations: Anti-CoV: Anti-Coronavirus; CNS: Central Nervous System; CSF: Cerebrospinal Fluid; EBV: Epstein-Barr Virus; HCoV-OC43: Human Coronavirus OC43; HSV: Herpes Simplex Virus; IgG: Immunoglobulin G; IgM: Immunoglobulin M; PCR: Polymerase Chain Reaction; RT-PCR: Reverse Transcription Polymerase Chain Reaction; SARS-CoV: Severe Acute Respiratory Syndrome Coronavirus; SARS-CoV-2: Severe Acute Respiratory Syndrome Coronavirus 2; VZV: Varicella Zoster Virus.

retrograde viral transport. ACE2 is highly expressed in enterocytes, likely serving as the target for SARS-CoV-2 to infect the gastrointestinal epithelium. Case reports have confirmed the detection of SARS-CoV-2 viral particles in enterocytes [32]. Since the vagus nerve functions as an important bridge in the gut-brain axis, retrograde axonal transport from the enteric neurons *via* the vagus nerve to the brainstem could explain SARS-CoV-2 neuroinvasion. To date, neither the enteric plexus nor the vagus nerve has been found to contain SARS-CoV-2.

Neurotropism of SARS-CoV-2

Complete genome analysis reveals that SARS-CoV-2 shares 79% nucleotide identity with SARS-CoV and 52% identity with MERS-CoV [33]. According to one computational model, the S protein of SARS-CoV-2 is quite similar to that of SARS-CoV, sharing a sequence identity of 77% [9]. The novel coronavirus demonstrates a 30% higher binding energy than SARS-CoV [9]. Several studies have confirmed that ACE2 is the binding receptor for SARS-CoV-2 [33-35] and the efficiency of its binding capacity is comparable to that of SARS-CoV [36]. Transmembrane protease serine 2 (TMPRSS2), responsible for S protein priming, is also crucial in the tropism of SARS-CoV-2 [37]. Therefore SARS-CoV-2 likely enters cells expressing ACE2 and TMPRSS2.

ACE2 is expressed throughout the body. The most enriched organs are the gastrointestinal tract, heart, kidney, lung and testis [38]. ACE2 in brain tissue has been implicated in the pathogenesis of Alzheimer's disease [39]. Using human pluripotent stem cells-derived neurons, Xu et al. [40] showed that ACE2 is robustly expressed in the neuronal cell bodies, with less expression in the axons and dendrites. Basal ganglia, cortex, hypothalamus, and substantia nigra have significant ACE2-associated expression quantitative trait loci, while the amygdala and cerebellum have fewer loci [41]. If ACE2 receptor expression is related to SARS-CoV-2 neurotropism, then it may result in selective vulnerability of particular brain regions.

However, the presence of ACE2 in neurons does not sufficiently prove the SARS-CoV-2 neurotropism. Previous pathologic studies and cell culture models have demonstrated that certain host cells with high ACE2 expression may afford low susceptibility to SARS-CoV, while other cells without ACE2 may be more vulnerable [42], underpinning the need for further investigation into the interaction between SARS-CoV-2 and its surrogate receptors.

Nevertheless, data remain limited for direct pathologic evidence of the neurotropism of SARS-CoV-2. RNAs of SARS-CoV-2 have been detected in the brain in an autopsy series of 27 COVID-19 patients [43]. Another study included 18 COVID-19 patients presenting with nonspecific neurological manifestations, with SARS-CoV-2 RNAs found by quantitative reverse transcription polymerase chain reaction (RT-PCR) in five patients. However, immunohistochemical analysis failed to show direct intrusion into the neurons or the glial cells [44]. To our knowledge, only two studies have found SARS-CoV-2 viral particles in brain tissues. One is reported in a patient with anosmia, dysgeusia and possible seizures. The authors observed numerous particles with the morphology compatible to that referable to virions of SARS-CoV-2 in the olfactory nerve, gyrus rectus and medulla oblongata [28]. The other study reported autopsy results in a patient with Parkinson's disease who developed confusion but no additional neurological symptoms. Post-mortem evaluation showed SARS-CoV-2 particles in neuronal intracytoplasmic vesicles in his frontal lobe. This was subsequently confirmed with molecular testing [7]. Although most neurologic complications in COVID-19 patients are likely secondary to indirect injury to the CNS (e.g., hypoxemia, electrolyte disorders, hypercoagulability), the paucity of confirmed post-mortem demonstration of SARS-CoV-2 in the CNS underscores the critical need for autopsy studies with careful neuropathologic assessment [45].

Neurovirulence of SAR-CoV-2

Hundreds of cases with neurological manifestations and dozens of cases of neurological disorders have been reported in patients with COVID-19 since the pandemic began [3, 46-49]. However, it is important to distinguish direct SARS-CoV-2 damage to the CNS from CNS manifestations due to the systemic physiologic abnormalities in COVID-19 patients. Some neurological manifestations (e.g., headache, altered consciousness) may be non-specific, yielding little value for screening and surveillance. For example, studies suggest cerebrovascular disease in COVID-19 patients is related to

down-regulation of ACE2 induced by SARS-CoV-2 with secondary overactivation of the renin-angiotensin system [50]. Confirmation of neurovirulence would require the detection of SARS-CoV-2 RNAs in the CSF, RNAs or antigens in brain tissues, or intrathecal synthesis of specific antibodies [51]. Among current case reports and reviews, few provide pathological or CSF evidence of SARS-CoV-2 (Table 1) [52]. Some authors propose a "three-step" infection model and argue that most neurologic complications occur in the last phase after the viral load has been significantly reduced by CNS clearance [53]. This model implies the need for CSF evaluation as early as possible in COVID-19 patients. Heterogeneous study quality further hinders evaluation of SARS-CoV-2 neurovirulence. For example, some studies included cases in which the presence of SARS-CoV-2 was suspected, but without providing confirmatory tests [28] or excluding other etiologies [54-57]. The presence of SARS-CoV-2 RNAs or viral particles does not provide incontrovertible evidence of neurovirulence [58]. Even when the virus is detected in the CSF, encephalitis still cannot be diagnosed without clinical or neuropathologic evidence of brain inflammation, letting alone the fact that the virus could be inactive causing no inflammation or acute disease [59]. Currently, there is not enough evidence to the support the notion that SARS-CoV-2 is a direct neurovirulent pathogen.

It is intriguing that there have been studies showing direct evidence of SARS-CoV-2 in the CNS in patients with neurological disorders. As illustrated in table 1, multiple cases of CNS infection have been reported [54-56, 60, 61]. Neurologic manifestations have included headache, seizure, encephalopathy and ischemic stroke, with rare reports of cerebellar symptoms and intracranial hemorrhage. All patients were found to be positive for SARS-CoV-2 by RT-PCR in the CSF, but not in nasopharyngeal specimens. In addition, acute necrotizing encephalopathy has been reported in a case with SARS-CoV-2 RNAs in the CSF. Importantly, the positive result was preceded by two negative ones, reinforcing the need for repeated CSF tests if the index of suspicion is high for SARS-CoV-2. There have been at least two cases of CNS demyelination with positive SARS-CoV-2 RNAs in patients' CSF [57, 62]. According to the case definition proposed by Ellul et al. [3], there is a probable association between the demyelinating disorder and SARS-CoV-2. Interestingly, both cases had the RT-PCR tests done weeks after the initial onset of COVID-19 and showed negative results in nasopharyngeal specimens, suggesting the virus persists in the CNS after clearance from the nasal cavity. In another study, viral particles were found in the gyrus rectus and brainstem in a patient with headache and possible seizure. However, this study lacked confirmatory identification of the viral particles, underscoring the need for consistent and detailed neuropathologic studies.

Pre-COVID-19 human coronaviruses appeared to be neurovirulent in long-term studies. One study showed that HCoV-OC43 can continuously cause pathology in mice even years after the initial acute CNS infection [63]. HCoV-OC43 and HCoV-229E have been discovered in patients with multiple sclerosis [17-21]. Interestingly, the 1918 H1N1 pandemic was paralleled by a mysterious epidemic of encephalitis lethargica followed by persistent parkinsonism [64] as has been demonstrated vividly in the movie "Awakenings". A recent study showed that acute H1N1 infection can hijack dopaminergic cells and disturb the cellular proteostasis, promoting the formation of α-synuclein and aggregation of Disrupted-in-Schizophrenia 1 [65]. Whether SARS-CoV-2 will also cause long-term CNS disorders might depend on its persistence in the CNS [59], the interaction with the host immune system [66] and its influence on cellular machineries [67]. Gomez-Pinedo et al. [59, 68] noted the absence of inflammation around the viral particles in brain tissues, suggesting the potential of the brain serving as a reservoir for SARS-CoV-2. The distribution of ACE2 is heterogeneous in the brain, with more expressed in the cortex and basal ganglia [41]. Accordingly, virus might selectively hide in these structures which is intriguingly consistent with the pathology of Parkinson's disease and Alzheimer's disease. Some authors proposed that SARS-CoV-2 might indirectly trigger neuronal inflammation, which will further contribute to the aggregation of pathological proteins [66]. Mechanisms similar to that seen with H1N1 have been proposed including endoplasmic reticulum stress, mitochondrial dysfunction, autophagy deficiency and loss of proteostasis [67]. The impact of the pandemic on neurodegenerative diseases can be multidimensional. The increased psychological stress induced by the pandemic might also play a role in the degeneration of dopaminergic cells and the discovery of latent hypokinetic syndrome [69].

Conclusions and Recommendations

Neurological complications have emerged as a significant cause of morbidity and mortality in the ongoing COVID-19 pandemic [70]. Given the accumulating evidence of neurological associated disorders in patients with COVID-19, it is critically important to gain greater understanding of the acute, chronic and potent delayed effects of the novel coronavirus on the nervous system. Although pathologic studies have demonstrated direct neuroinvasion *via* hematogenous spread and retrograde transport by the olfactory nerve, the underlying pathophysiology remains elusive. Retrograde axonal transport through the vagus nerve and transcribrial route are additional potential mechanisms for neurotropism. ACE2 is believed to be the receptor for SARS-CoV-2, but a detailed understanding of the mechanism of its neurotropism requires further investigation. Currently, limited clinical and pathologic evidence supports SARS-CoV-2 as a neurotropic and neurovirulent pathogen. More direct evidence of the novel coronavirus in CSF and brain parenchyma should be sought. Given the possibility of the coronavirus becoming undetectable in the CNS, prospective surveillance for long-term sequelae in COVID-19 patients is warranted [70].

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